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PREPARATION OF HIGLY POROUS HYDROXYAPATITE CERAMICS FROM CUTTLEFISH BONE

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Abstract: Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, has been widely investigated for medical purposes because it is suitable for either direct clinical use as implants or tissue-engineering applications. In this work materials were successfully produced via hydrothermal transformation of aragonite, obtained from fresh cuttlefish bones, at 200°C and high pressures. Beyond low production cost, worldwide availability and natural–biological origin of raw materials, the produced materials have ideal pore size and interconnectivity features suitable for supporting biological activities, such as bone tissue growth and vascularization. The cuttlefish bone (*Sepia officinalis L.)* from Adriatic Sea was used. The samples of cuttlebone were pretreated, boiled with NaClO in order to remove the organic phase of the bone to accelerate the conversion. The substitution of CO_3^{2-} groups predominantly into the PO_4^{3-} sites of the HAP structure was determined by FTIR spectroscopy. SEM micrographs have shown that the interconnected hollow structure with pillars connecting parallel lamellae in cuttlefish bone is maintained after conversion. Specific surface area (S_{BET}) and total pore volumes increased and mean pore size decreased by high temperature treatment.

Keywords: Scaffold, Cuttlefish bone, Hydroxyapatite, Microstructure.

Introduction

Bioceramics are a group of ceramic materials that are specially developed for use as medical and dental implants. An important member of this group is hydroxyapatite /$Ca_{10}(PO_4)_6(OH)_2$/. It is the main inorganic component of hard tissues of the bones, and it accounts for 60-70% of the mineral phase in the human bone. Due to its similarity with the mineral phase of bone has been studied for many years as implant material [1]. In recent years, particular attention was paid to the synthesis of Hap ceramic with porous morphology required for vascularization, bone cell invasion, and angiogenesis, which further improve its biomedical properties. Artificial implants of Hap are very popular for hard tissues restorations because they accelerate bone growth around the implant. Such materials should have porosity and compression strength similar to the tissues they will substitute, in order to assure bioactivity and biocompatibility [2]. The use of natural inorganic structures and materials for medical purposes has been motivated by limitations in generating synthetic materials with the requisite structure and mechanical integrity. The hydrothermal method for hydroxyapatite formation directly from corals was first used by Roy and Linnehan [3] in 1974. They reported that complete replacement of aragonite by phosphates was achieved at 270°C and 103 MPa. Kasperk and Ewers [4] prepared hydroxyapatite from calcified algae in 1986. Tkalcec et al. [5] prepared Hap hydrothermally from Adriatic shells Arca Noae in 1989. HAp derived from Indian coral using hydrothermal synthesis was developed by Sivakumar et al. in 1996 [6]. However, the resultant materials were in the form of powder and required further forming and shaping. Scaffolds of HAp from cuttlefish bones via hydrothermal transformation were first synthesized in 2005 [7].

The aim of the present work is to study the hydrothermal conversion of the aragonite (calcium carbonate) of cuttlefish bones, which is structured to combine high compressive strength with high porosity and large pore dimension, into HAp retaining its overall structure after conversion.

Materials and experimental procedure

Bone of cuttlefish (Sepia Officinalis L.) from Adriatic sea were gently cut in small pieces (about 2 cm^3) and boiled with 4% NaClO in order to remove the organic component. The samples were then sealed with the 15 mL aqueous solution of 0.6M $(NH_4)_2HPO_4$ (stoichiometrical amount) in teflon lined stainless steel pressure vessel at 200 °C for various times (1 to 48 hours). The converted HAp was washed with boiling water and dried at 110 °C. The micro-structure of boiled and hydrothermal treated (HT) cuttlefish bones were examined by scanning electron microscopy (SEM ISIDS-130). The conversion of aragonite into HAp was followed by X-ray diffraction analysis (Philips PW 1820 counter diffractometer with monochromatized Cu Kα radiation). Fourier transform infrared spectra (FTIR) were performed by attenuated total reflectance (ATR) spectroscopy for solids with a diamond crystal. Specific surface area and mean pore size were determined by nitrogen adsorption-desorption isotherms at liquid N_2 temperature on a Micromeretics ASAP 2000 instrument. The mean pore size was estimated from the desorption branch of the nitrogen adsorption-desorption isotherms by the Barrett-Joyner-Halenda (BJH) method.

Results and discussion

The specific surface area, mean pore size and total pore volume of raw cuttlefish bones and converted Hap is shown in Table 1. The monoliths about 1 cm^3 large were used for the analysis. The specific surface area, S_{BET}, of raw material is 2.5 m^2g^{-1}. When the samples were boiled with NaClO, incomplete removal of organic component reduced it to 0.6 m^2g^{-1}, but after hydrothermal treatment it increased to 9.9 m^2g^{-1}.

The conversion of aragonite into hydroxyapatite was followed by XRD analysis (Fig. 1) and FTIR spectroscopy (Fig. 2). The raw cuttlefish bone comprises pure aragonite which gradually converts into HAp by treatment with $NH_4H_2PO_4$ under hydrothermal conditions described above. The positions of aragonite lines correspond to JCPD file No 41-1475. The product after hydrothermal treatment was identified to be hydroxyapatite (JCPDS 09-0432).

Table 1. Specific surface area, mean pore size and total pore volume of raw cuttlefish bone and hydrothermally converted Hap

Cuttlefish bone	S_{BET} (m^2g^{-1})	Mean pore size (nm)	Total pore[a] volume (cm^3/g)
Raw	2.5	20	0.0134
Boiled with NaClO	0.6	26.5	0.0039
HAp 200°C/24 h	9.9	19.2	0.0489

[a] estimated using BJH desorption branch of the isotherms.

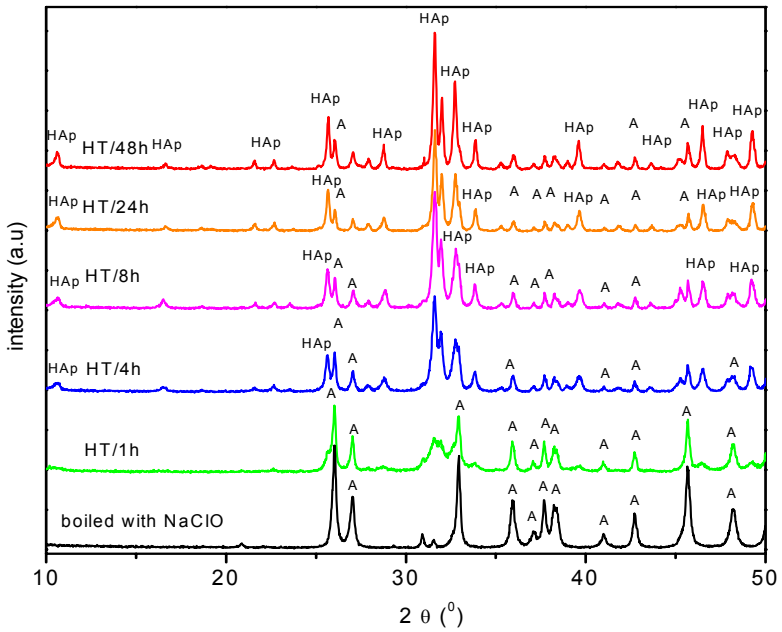

Fig. 1. XRD patterns of hydrothermally (HT) converted HAp at various times (1-48 h). The A and HAp symbols are the 2θ positions of aragonite (JCPDS 41-1475) and hydroxyapatite (JCPDS 09-0432).

XRD analysis showed that the amount of HAp increased with time but the complete conversion has not been reached even after 48 h.

In FTIR spectrum of cuttlefish bone boiled with NaClO (Fig. 2) only absorption bands of CO_3^{2-} functional groups in aragonite were seen (871, 1632, 1547, 1455, 712, 710 cm^{-1}) [8]. HT treatment at 200 °C for 1 hour caused the appearance of the bands assigned to PO_4^{3-} groups (ν_3—1085 and 1010 cm^{-1}; ν_1—960 cm^{-1}; ν_4—558 and 597 cm^{-1} and ν_2—473 cm^{-1}) The bands attributed to OH^- groups (631 cm^{-1}) and CO_3^{2-} groups substituted for PO_4^{3-}, respectively, (871 cm^{-1}, 1454 and 1414 cm^{-1}), [9] were seen in sample after HT treated at 200 °C for 8 hours. The intensities and the resolution of bands increased with the time of HT treatment. In sample treated for 48 hours the band at 1544 cm^{-1} due to the CO_3^{2-} groups substituted for OH^- [10] were also observed.

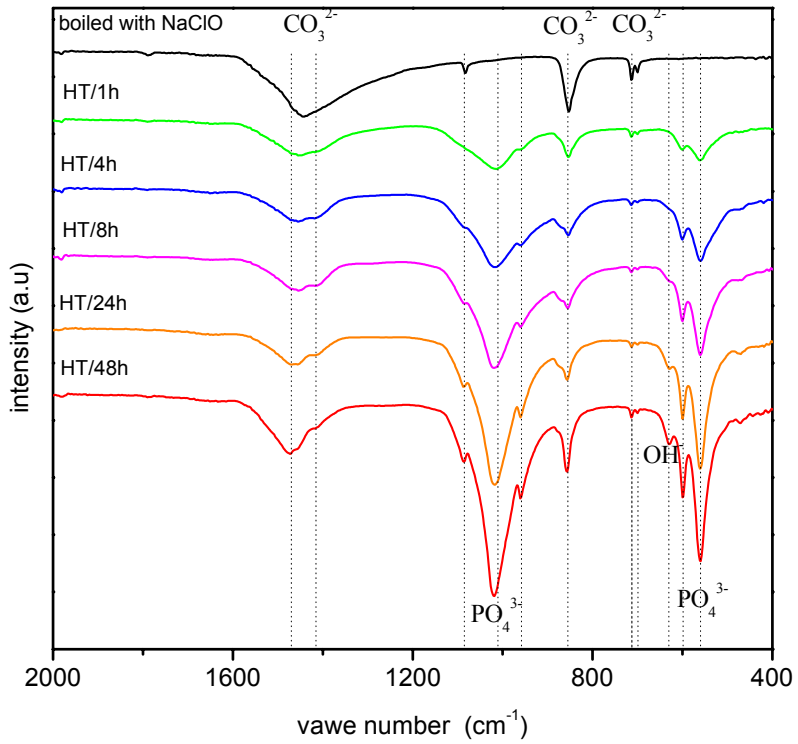

Fig. 2. FTIR spectra of cuttlefish bones boiled with 4% NaClO and hydrothermally transformed in HAp at various conditions.

Microstructure of the cuttlefish bone boiled with NaClO is shown in Fig. 3(A). The organic material enveloped the bones is partially removed by boiling. The cuttlebone (which accounts about 9 % of the animal's volume is a hollow structure, divided by parallel sheets (lamellae) which form chambers sealed from each other. The spacing of the lamellae is about 200 μm, as seen in Fig. 3(A), but it varies in different areas of the cuttlebone; usually between 200 and 600 μm. The lamellae are supported by numerous pillars which have sigmoidal cross-section. The higher magnification (insert) shows that the columns have a corrugated appearance with pore size in nanometer range.

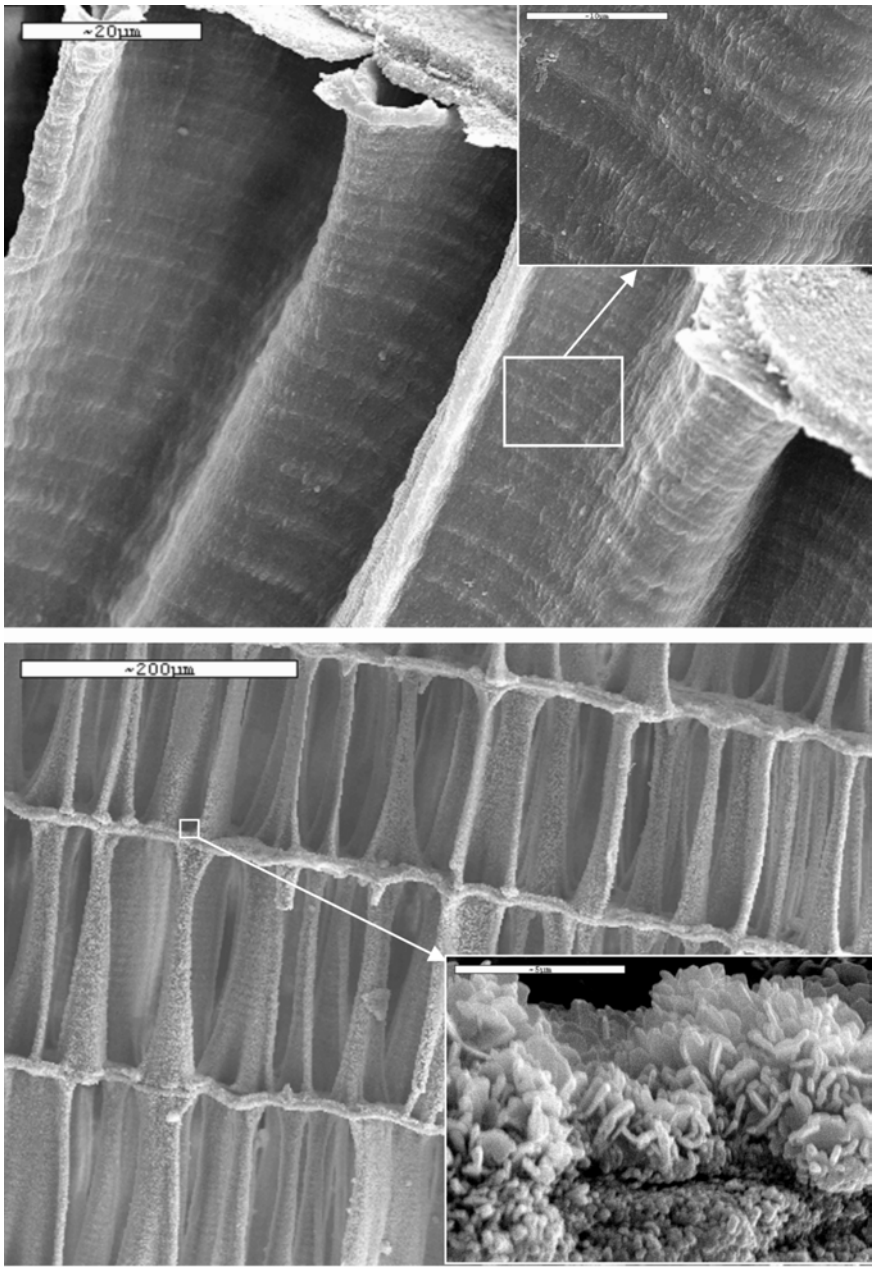

Fig. 3. SEM micrographs of (A) cuttlefish bone boiled with NaClO. The corrugated appearance of the pillars is shown in the insert. (B) the cuttlefish bone after hydrothermal conversion at 200°C/24 h showing the plate – and needle – like HAp crystals (insert). The conversion did not complete and on the surface of lamellae there is still aragonite structure.

The corresponding micro-structure converted HAp is shown in Fig. 3(B). The same hollow interconnected structure of cuttlefish bone is retained during the HT conversion. As seen at higher magnification (insert) the plate- and needle-like HAp crystals are formed. The microstructure explains the high porosity of cuttlefish bones measured by mercury porosimeter (about 90%) and is in agreement with literature [10]. the increase of S_{BET} and total pore volume decreasing the mean pore size (Table1).

Conclusion

Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, has been produced via hydrothermal treatment. Aragonite samples ($CaCO_3$) from cuttlefish bone were transformed into HAp by hydrothermal reaction with $NH_4H_2PO_4$ inside sealed autoclaves at 200°C for various times (1-48 hours). FTIR spectra show the characteristic bands of Hap. Hydrothermal conversion retains the interconnected channeled structure of cuttlefish bone while altering the chemical composition from aragonite to hydroxyapatite. Plate- and needle-like crystals of HAp were formed on the surface of lamellae but there is still easy to detect aragonite structure because the reaction did not complete under specific conditions. The specific surface area (S_{BET}) and total pore volume is increased and mean pore size decreased by HT treatment.
The porosity of the cuttlefish bones is measured by mercury porosimetry to be about 90 wt% and is in agreement with literature [10].

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